Aust. J. Biol. Sci., 1975, **28**, 85–88

Studies on Ovine Infertility in Agricultural Regions of Western Australia: Plasma Progesterone Levels of Fertile and Infertile Ewes

J. F. Smith

Department of Animal Science and Production, University of Western Australia, Nedlands, W.A. 6009; present address: Ruakura Animal Research Station, Private Bag, Hamilton, New Zealand.

Abstract

No differences were observed in the levels or patterns of production of progesterone between fertile ewes and ewes with the 'permanent' type of infertility due to ingestion of phyto-oestrogens.

Introduction

Infertility in sheep due to the ingestion of oestrogenic pastures can be of two types: 'temporary', which is completely reversible (Morley *et al.* 1966) and manifest only when ewes are consuming phyto-oestrogens at the time of breeding; and 'permanent', in which the ewe remains infertile regardless of the type of food being ingested at the time of breeding (Bennetts *et al.* 1946). There is poorer sperm transport in ewes affected with the permanent-type infertility and subsequently lower fertilization than in control ewes (Lightfoot *et al.* 1967; Kaltenbach and Davies 1970).

In both forms of infertility the length of oestrus is identical in affected and control ewes (Lightfoot *et al.* 1967; Obst and Seamark 1970). However, Obst and Seamark (1970), who studied the temporary form of infertility, showed that secretion of progesterone stopped earlier in ewes eating oestrogenic clover than in control ewes.

The purpose of the work presented here was to determine whether sheep affected with the permanent type of infertility have an altered pattern of progesterone production.

Methods

Experimental Animals

Two groups of six Merino ewes of the same age (5–6 years old) were obtained from a property that has a 'dry ewe' problem and history of clover disease. All ewes had lambed at least once up to 1968. The ewes designated 'fertile' had produced lambs in 1968, 1969, and 1970 while those designated 'infertile' had failed to produce lambs in those years. The 12 ewes were run together with two vasectomized rams and all were maintained on a non-oestrogenic diet.

Collection of Samples

All ewes had Silastic catheters inserted into the jugular vein and blood samples were obtained at 10.00 a.m. on the day of oestrus and daily until the next oestrus. The blood samples were centrifuged and the plasma frozen immediately after collection and stored at $-14°C$ until they were assayed.

Progesterone Assay

Assays of all samples were in duplicate. Plasma (1·0 ml) was heated to 60°C for 10 min to denature any transcortin present. Extraction with 5 volumes of light petroleum was accomplished

by mixing for 10 min on a horizontal shaker. The organic phase was transferred to a glass tube (75 by 10 mm) and evaporated to dryness. The residue was dissolved in 1 ml of a protein-binding solution, containing 2·0 μCi [1,2-^{3}H]corticosterone and 1·0 ml laying hen plasma in 100 ml distilled water, and mixed on a vortex mixer. Tubes were incubated at 40°C for 10 min and at 4°C for a further 30 min. To each tube was added 60 mg Florisil, and the resulting mixture shaken for exactly 30 s on a vortex mixer and returned to an ice-bath for 5 min.

An aliquot of the supernatant (0·5 ml) was transferred to a counting vial containing 10 ml of Triton scintillation mixture and counted to a preset count. Reference samples containing 0, 0·25, 0·5, 1·0, 2·5 and 5·0 ng progesterone were included in each assay. The percentage bound [1,2-^{3}H]-corticosterone was plotted against amount of progesterone added. The progesterone content of the unknown samples was calculated from the standard curve based on the reference samples; the results were corrected for procedural losses (Murphy 1967).

Results

Assay

Because no chromatographic step was included in the assay there could have been some loss of specificity. There was less than 2% recovery of labelled cortisol following the light petroleum extraction. Accuracy was assessed by the addition of progesterone to a bulk plasma pool and this was assayed as described. The percentage recovery varied from 90 to 105% over the range 0·5–2·5 ng. The reproducibility of the method was determined by estimating the standard deviation of duplicate determinations. Over the range 0·5–10·0 ng the coefficient of variation ranged from 8 to 12% (n = 180). Taking procedural losses into account the practical limit of detection was 0·1 ng/ml.

Cycle Length

The mean cycle length for the fertile ewes was 17·3 (S.D. ±0·6) days and for the infertile ewes 17·5 (S.D. ±0·4) days.

Progesterone Levels

The patterns of progesterone concentration found are shown in Fig. 1. There was no difference between the fertile and infertile ewes.

Low levels of 0·2–0·5 ng/ml were recorded at oestrus and levels rose to 3–4 ng/ml by day 5. This level was maintained until day 15 of the cycle after which the concentration fell rapidly to less than 1 ng/ml on day 16.

Discussion

The similar cycle length of both groups confirms the findings of Lightfoot *et al.* (1967).

The overall pattern of progesterone levels is similar to that reported by Thorburn *et al.* (1969). However, the levels observed in the present trial are considerably higher in the mid-luteal phase than those reported by Thorburn *et al.* (1969) and Obst and Seamark (1970). This may be due to the different assay technique since Florisil sometimes gives results up to twice as high as those obtained with Sephadex (K. P. Croker and C. Jaume, personal communication).

It is quite clear from these results that differences in the amounts of cervical mucus produced by fertile ewes and ewes affected with permanent clover disease cannot be attributed to differences in the amount or pattern of production of progesterone.

Smith (1971) suggested that the poor transport of sperm may be due to the amount of cervical mucus produced by ewes affected with permanent clover disease, which is much greater than that produced by fertile ewes. It is quite clear from the results presented above that these differences in amount of cervical mucus produced cannot be attributed to differences in the amount of progesterone produced or its pattern of production.

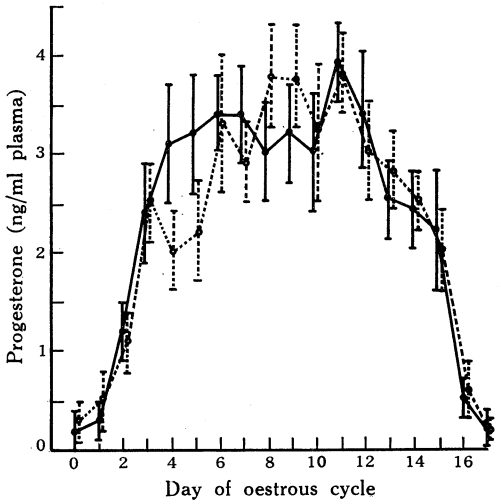

Fig. 1. Plasma progesterone concentration during the oestrous cycle of fertile (○ - - - - ○) and infertile (●——●) ewes. Each point represents the mean (±S.D.) for six ewes.

The evidence of Smith (1971) indicates that the sensitivity to exogenous oestrogen, as measured by amount of cervical mucus produced, is similar for both fertile and infertile ewes. While the duration of oestrus, which is similar for both groups, may not be as sensitive to changes in endogenous levels of oestrogen as is the amount of cervical mucus produced, if the differences in the amount of cervical mucus reported by Smith (1971) were due solely to differences in levels of oestrogen the magnitude of these would be sufficient to produce differences in the duration of oestrus (Fletcher and Lindsay 1971; Kelly 1973). As no such changes were observed by Smith (1971) it is difficult to find a ready explanation for the differences in the amount of cervical mucus produced. Elucidation of this problem is dependent on the assay of endogenous oestrogen levels in both groups of animals.

This notwithstanding, the results show an important endocrinological difference between the temporary and permanent types of phyto-oestrogen infertility in that the temporary form is marked by a change in progesterone production (Obst and Seamark 1970) but this is not the case in the permanent form studied here.

This difference is most probably due to the difference in diets between temporary and permanent infertile ewes at the time of sampling. The premature decline in progesterone levels associated with the temporary form of infertility could be due to a possible effect, either directly or indirectly, of ingested phyto-oestrogens on the corpus luteum.

Acknowledgments

I would like to thank Dr D. R. Lindsay for his advice and criticism in the course of this work. Technical assistance was provided by Miss W. de Boar and Mr T. Johnson.

The work was supported by funds from the E. H. B. Lefroy Research Fellowship in Agriculture.

References

Bennetts, W. H., Underwood, E. J., and Shier, L. F. (1946). A specific breeding problem of sheep on subterranean clover pastures in Western Australia. *Aust. Vet. J.* **22**, 2.

Fletcher, I. C., and Lindsay, D. R. (1971). Effect of oestrogen on oestrous behaviour and its variation with season in the ewe. *J. Endocrinol.* **50**, 685.

Kaltenbach, C. C., and Davies, H. Lloyd (1970). Fertilization, sperm transport, and early embryonic loss in ewes grazed on cultivars of subterranean clover (*T. subterraneum*). *Aust. J. Agric. Res.* **21**, 107.

Kelly, R. W. (1973). Metabolism and physiological action of phyto-oestrogens in sheep. Ph.D. Thesis, University of Western Australia.

Lightfoot, R. J., Croker, K. P., and Neil, H. G. (1967). Failure of sperm transport in relation to ewe infertility following prolonged grazing on oestrogenic pastures. *Aust. J. Agric. Res.* **18**, 755.

Morley, F. H. W., Axelsen, A., and Bennett, D. (1966). Recovery of normal fertility after grazing on oestrogenic red clover. *Aust. Vet. J.* **42**, 204.

Murphy, B. E. P. (1967). Some studies on the protein-binding of steroids and their application to the routine micro- and ultra-micro-measurement of various steroids in body fluids by competitive protein-binding radio assay. *J. Clin. Endocrinol. Metab.* **27**, 973.

Obst, J. M., and Seamark, R. F. (1970). Plasma progesterone concentrations during the reproductive cycle of ewes grazing Yarloop clover. *J. Reprod. Fertil.* **21**, 545.

Smith, J. F. (1971). Studies on ovine infertility in agricultural regions of Western Australia: cervical mucus production by fertile and infertile ewes. *Aust. J. Agric. Res.* **22**, 513.

Thorburn, G. D., Bassett, J. M., and Smith, I. D. (1969). Progesterone concentration in the peripheral plasma of sheep during the oestrous cycle. *J. Endocrinol.* **45**, 459.

Manuscript received 14 January 1974